Measurement of Sunscreen Immune Protection Factors in Humans: A Consensus Paper

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It is increasingly accepted that sunscreens should protect against ultraviolet radiation (UVR)-induced immunosuppression, with an index of protection that can be compared with the sun protection factor (SPF). Five groups of immunoprotection researchers met to discuss the status of immune protection factor (IPF) evaluation in human skin *in vivo*. Current methods rely on a suncreen's inhibition of UVR-induced local suppression of the contact hypersensitivity (CHS) response or the delayed-type hypersensitivity (DTH) response, using either the induction or the elicitation arms of these responses. The induction arm of the CHS response has the advantage of being sensitive to a single sub-erythemal exposure of solar-simulating radiation (SSR) that allows a direct comparison with the SPF. This approach, which necessitates sensitization, requires a large number of volunteers and is too labor intensive and time consuming to become a routine method. The elicitation arm of the CHS or DTH responses exploits prior sensitization to contact or recall antigens and has the advantage of being possible to apply on small groups of volunteers. Some current protocols, however, require repeat SSR exposures, which invalidates a direct comparison with SPF that is based on a single exposure. There is a need for a new simpler method of IPF that will have to be validated against existing models.

Key words: consensus paper/immune protection/sunscreens/ultraviolet radiation
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Human ultraviolet radiation (UVR)-induced immunosuppression (Cooper *et al*, 1992; Kelly *et al*, 1998, 2000) probably plays a role in skin cancer (Nishigori *et al*, 1996; de Gruijl, 2002; Ullrich, 2002). The standard method of assessing sunscreen protection is based on erythema and is expressed as the sun protection factor (SPF). It is recognized that labelled SPF is often not achieved, because users typically apply sunscreens at lower application densities than the 2 mg per cm^2 required by regulatory bodies (Diffey, 1996). Quite apart from the behavioral issues that determine the actual SPF achieved from a product, erythema is a poor indicator of immunosuppression (Kelly *et al*, 2000). This raises the question: "Is the immune protection factor (IPF) of a sunscreen equivalent to its SPF?" Several studies (e.g., Ullrich *et al*, 1999; Cooper et al, 2002) indicate that sunscreens afford some protection against UVR-induced immunosuppression, but wide variations in experimental design and data management make it difficult to standardize the assessment and definition of IPF. An expert panel (Table I), convened by L'Oréal Recherche in Paris on 5th of July 2002, discussed these issues, which form the basis of this paper.

Abbreviations: CHS, contact hypersensitivity; DNCB, dinitrochlorobenzene; DTH, delayed-type hypersensitivity; EI, erythema index; ID_{50}, UVR dose that results in 50% immunosuppression; IPF, immune protection factor; MED, minimal erythemal dose; MISD, minimal immunosuppressive dose; PAR, primary allergic response; SFT, skin fold thickness; SPF, sun protection factor; SSR, solar-simulated radiation; ts, total score; UVR, ultraviolet radiation

Immunological Background to IPF Methodology

UVR suppresses the induction and elicitation arms of the contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses. CHS is a response to topically applied antigens, whereas DTH is the reaction to intracutaneously delivered antigens. Exposing naïve volunteers to UVR before antigen sensitization assesses suppression of the induction arm. This assessment is made by challenge with the same antigen 2–3 wk later. The assessment of the suppression of the elicitation arm is made on volunteers with prior sensitization via vaccination or environmental exposure to common contact allergens such as nickel. In this case, the volunteers are exposed to UVR and re-challenged with the relevant antigens or contact allergen. Failure to induce or elicit sensitization by applying or delivering the antigen to the UVR-exposed site is called local immunosuppression and failure to induce or elicit sensitization by applying or delivering the antigen to a non-UVR exposed distant site is called systemic or distal immunosuppression.

Table I. Investigative groups and techniques used to assess immunoprotection

Group	Techniques used for IPF assessment	Relevant references
Australian	Suppression of elicitation response to nickel CHS	Poon *et al* (2003)
Austrian	Suppression of sensitization to DNCB	Wolf *et al* (2003)
French	Suppression of elicitation response to recall DTH	Moyal and Fourtanier (2003)
UK	Suppression of sensitization to DNCB	Kelly *et al* (2003)
USA	Suppression of sensitization to DNCB	Baron *et al* (2003)

DNCB, dinitrochlorobenzene; IPF, immune protection factor; CHS, contact hypersensitivity; DTH, delayed type hypersensitivity.

The IPF of a sunscreen has been evaluated using the induction or elicitation arm of the local CHS or DTH response (see Table I), and the systemic DTH response (Moyal and Fourtanier, 2001).

Methodology Used by the Five Groups

Sunscreens It is important to characterize the sunscreens studied. Apart from their names and concentrations, actives such as antioxidants should be noted as these may influence UVR-induced immunosuppression. Sunscreen absorption spectra should be determined spectrophotometrically *in vitro*, using either a Transpore tape (3M, Reuil, Malmaison, France) (Diffey and Robson, 1989) or a roughened quartz plate (Moyal and Fourtanier, 2001) as a substrate. Such spectral data enable the calculation of different *in vitro* factors such as SPF and critical wavelength (λ_c) (Diffey, 1994).

It is very important to verify the *in vivo* assigned SPF because this depends on the solar-simulating radiation (SSR) source used for its assessment (LeVee *et al*, 1980), as well as the method of sunscreen application and the clinical evaluation of erythema. In all cases, the same SSR source and similar volunteers (phototype, age range, sex ratio, and body site) as those included for IPF assessment should be used. Furthermore, the same investigator should apply the sunscreen for SPF and IPF assessments. The European Cosmetic Toiletry and Perfumery Association (Colipa, 1994) or the Food and Drug Administration (FDA, 1999) recommendations should be followed for SPF determination. Erythema intensity can be assessed clinically or quantified using colorimetric measurements (Chromameter Minolta, Osaker, Japan; Diastron Dia-stron, Andover, Oklahoma) (Colipa, 1994).

UVR sources and dosimetry SSR spectrum must comply with a standard for SPF determination (e.g., Colipa, 1994). It is important to measure SSR spectral irradiance at skin level with a calibrated spectroradiometer. Routine spectroradiometry is time consuming so calibrated broadband radiometers are usually used for day-to-day measurements.

Volunteers and test sites Inclusion criteria, such as sex, skin type, and test site, may influence the results. Immunity decreases with age but the effect of age on photoimmunosuppression is unknown. The volunteers in the studies of Table I ranged from 18 to 71 y old (UK: 18–35, Austria: 18–60, France 18–40, Australia 18–71, USA: 18–60). Menstruating women undergo marked monthly fluctuations in their immune responsiveness but at mid-cycle, their immune response is similar to men (Oberhelman *et al*, 1992). The UK and US groups sensitized females at mid-cycle to control for this. The Austrian and French groups dropped females from the sunscreen IPF testing because of variability in response observed in the first part of their study. It is easier to find females for nickel elicitation studies because more women (15%) than men (5%) develop allergic dermatitis to nickel. The UK group has shown that susceptibility to immunosuppression (induction of CHS) is skin type dependent (Kelly *et al*, 2000), with skin types I/II being more readily suppressed than skin types III/IV. But the Australian group found no relationship between susceptibility to sunburn, which is roughly skin type dependent, and susceptibility to suppression of the elicitation of CHS to nickel (Damian *et al*, 2001).

All groups, except the Australians, assessed individual sensitivity to SSR by determination of the minimal erythemal dose (MED) 48 h to 2 wk before the immune function assays. The MED is the SSR dose (J per m^2) required to induce a just visibly perceptible erythema or an erythema with well-defined borders 24 h after exposure. The buttock was the sensitization site for induction of CHS studies because this area is relatively flat with an even color and, in general, UVR naïve. The buttock is not suitable, however, for DTH because of its softness makes it difficult to give a homogeneous intracutaneous delivery of allergens. In this case, the back is preferred, which was also used for the elicitation of CHS to nickel because it offers a larger flat area than buttock skin. For induction studies, the challenge (or elicitation) was always performed on the UVR-protected upper inner arm, opposite to the UVR-exposed site (left arm when right buttock).

It is important that both SPF and IPF determinations are based on a set of homogeneous volunteers with the same inclusion criteria. Moreover, for IPF determination in elicitation studies, the initial immune response of the volunteers has to be considered for randomization of different groups, and for selection of nickel concentrations used in the challenge patches. It is important to note that comparisons between IPF and SPF must be made in the same anatomical sites.

SSR doses and group design In general, the group size was 6–15 volunteers. All groups except one (Australian) based SSR doses on individual MED, giving fractions or multiples of the individual MED that were determined prior to the immunological protocols. Single SSR exposure protocols used doses between 0.25 and 3 individualized MED on unprotected sites. In repeated exposure protocols, which assessed the suppression of nickel-induced CHS, all volunteers received the same SSR doses that were not greater than 1 mean MED per exposure. This limits the SSR-induced erythema, which could otherwise interfere with the assessment of CHS. The mean MED was determined on a different but comparable group of volunteers.

The sunscreen-treated sites received MED increments that were comparable with unprotected skin after the test

product SPF had been taken into account, this being 0.25–3 MED × SPF. In general, the SPF was previously determined on a separate group of comparable volunteers. But, the US group determined the individual SPF, and the SSR dose given to the sunscreen-treated individuals was calculated using individual SPF.

With the exception of the Australian group, volunteers were randomly assigned into either: SSR + sunscreen or SSR-only groups. In addition, control groups were included such as no treatment at all, SSR exposed but non-sensitized, non-SSR-exposed but sunscreen treated, and challenged only (for induction study). For the DTH elicitation study, subjects allocated to the different groups were selected based on their initial DTH responses. In the nickel elicitation studies (Australian group), each volunteer received a range of SSR doses with and without sunscreen as well as control treatments (non-exposed-untreated, non-exposed-sunscreen treated).

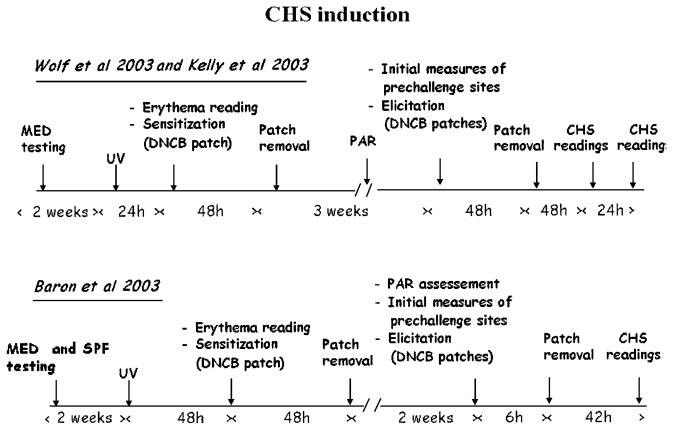

Figure 1
Study protocols used by Austrian, UK and USA groups for evaluation of solar-simulating radiation (SSR) suppression of the induction arm of contact hypersensitivity (CHS) response.

Immunological protocols to assess IPF

SSR suppression of the induction arm of the immune response The Austrian, UK, and US groups used the CHS response to a topically applied chemical hapten, dinitrochlorobenzene (DNCB). Their protocols were very similar, as shown in Fig 1. The hapten was dissolved at 31.2 μg per 50 μL (0.0624%) either in ethanol (UK, Austria) or acetone (US). The response to subsequent challenge was assessed on five patches placed on the test site with incremental doses of DNCB (generally from 3.125 to 25 μg per 20 μL (0.015625%–0.125%) with a geometric progression) and a vehicle control.

Erythema on the buttock IPF test site, exposed with or without sunscreen, was assessed clinically and/or by colorimetry before sensitization with DNCB. The sensitization was performed with a patch (*in situ* for 48 h) 24–48 h after SSR exposure. The challenge on the inner arm, 2 or 3 wk after sensitization, was with DNCB patches that were applied to each volunteer between 6 and 48 h. The elicitation responses were measured 48–72 h after patch removal. The primary allergic response (PAR) on the sensitization site was always assessed, usually about 2 wk after the removal of the initial DNCB patch. The severity of the PAR is an indicator of the likely intensity of the elicitation response (Kelly *et al*, 1998), and a strong PAR results in the exclusion of a volunteer or a reduction of the DNCB challenge concentrations to prevent severe blistering.

SSR suppression of the elicitation arm of immune response Two groups used the elicitation arm of the immune response (See Fig 2). The French group used a commercial kit (Multitest kit Pasteur Mérieux, Lyon, France) that contained seven antigens (tetanus toxoid, diphteria toxoid, *Streptococcus*, tuberculin, *Candida albicans*, *Trichophyton mentagrophytes*, and *Proteus mirabilis*) encountered in childhood immunization and their vehicle (70% sterile glycerine). These antigens are delivered intracutaneously, so they induce DTH reactions. The Australian group used nickel (nickel sulfate), which is a common contact allergen, that was topically applied in a petrolateum base to elicit the CHS response.

The French group assessed DTH responses 48 h after challenge. Baseline immunity 1 wk prior to SSR exposure was determined in each volunteer. The volunteers were then exposed to SSR (based on MED previously assessed) and were re-challenged 24 h later. In addition, the erythemal response on the exposed sites, with or without sunscreen, was evaluated by clinical scoring or/and colorimetry before challenge. The Australian group enrolled nickel-sensitive volunteers and measured the CHS response 24 h after the removal of nickel patches that were left *in situ* for 48 h. In this study, each volunteer was exposed to the same SSR dose for 4 consecutive days. Two lots of nickel patches were applied on each volunteer. The first lot was on the first day of irradiation at a site distant from the test area. This was to assess the initial CHS response and select the nickel concentration for IPF assessment. The second lot of patches was applied on the final day of SSR exposure. Erythema induced by repeated sub-erythemal exposure (with or without sunscreen) was assessed at the same time as the nickel response, 72 h after the last SSR exposure.

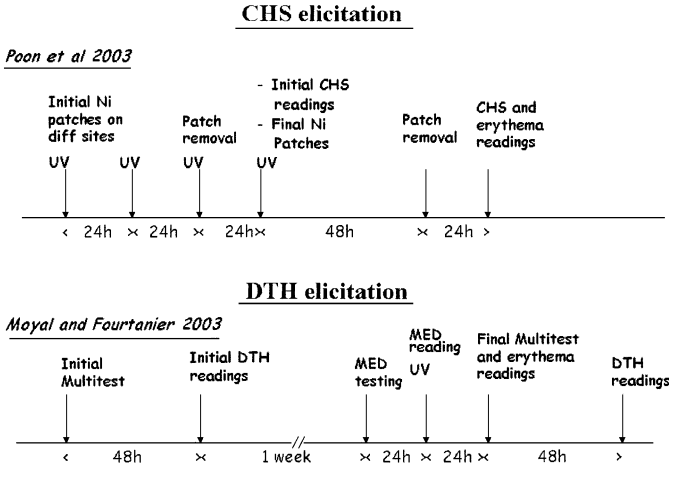

Figure 2
Study protocols used by Australian and French groups for evaluation of solar-simulating radiation (SSR) suppression of elicitation arm of the contact hypersensitivity (CHS) or delayed type hypersensitivity (DTH) response.

Quantification of responses

Suppression of induction Subjective (clinical score) and quantitative methods were used to assess the CHS response and the results were expressed in different ways. The UK group determined the percentage increase in dermal thickness by 20 MHz ultrasound image analysis and scans were performed immediately before, and 48 and 72 h after the challenge. The slope of linear regression analysis of these measures represented the CHS response of a given volunteer, in other words, the slope of the DNCB dose–response. The Austrian group also took ultrasound measurement but in addition measured the reaction diameter. They used the average CHS response of all elicitation sites for a given volunteer, as they had previously observed a linear relationship between CHS response and DNCB concentrations. The US group determined the increase in skinfold thickness (SFT) at each elicitation site, and an overall score per volunteer was given by the sum of the SFT of all elicitation sites. Each challenge site was measured before and after patch application. In addition, the three groups visually scored the CHS responses. The US group used the North American Contact Dermatitis Group system (Baron *et al*, 2003). The Austrian (Wolf *et al*, 2003) and the UK (Kelly *et al*, 1998) groups used their own defined grading systems.

Suppression of elicitation The French group summed the mean diameter of each positive reaction to each recall antigen to obtain a total score (TS) for each volunteer. Reaction borders were defined by redness and induration. The Australian group quantified the CHS response to nickel by determining an erythema index (EI) by reflectance spectroscopy. After subtraction of the background (absence of nickel patch), the EI of the exposed sites (with or without sunscreen) were compared with the EI of the appropriate unirradiated nickel-patched sites. The results are expressed as $\Delta EI = EI\ (\text{unirradiated control}) - EI\ (\text{test site})$.

IPF calculation

Suppression of induction studies The IPF was derived from the CHS responses of all sensitized volunteers. IPF was either considered as the ratio, between unprotected and protected groups, of a particular endpoint such as the SSR dose that induced 50% immunosuppression (ID_{50}), or as the ratio of the two SSR dose–responses-modelled curves for CHS responses with and without sunscreen. In both approaches, the dose–response relationship between individual CHS response and the dose of SSR given were modelled for both unprotected and protected groups with the same nonlinear regression models. Generally, two-parameter exponential (1) or logistic curves (2) were fitted, based on the following types of equations:

$$-y = c \times \exp\left(-b\frac{\text{dose}}{\text{IPF}}\right) \quad (1)$$

where y is the immune response, IPF equals 1 for unprotected groups, $b > 0$, and c is the maximal immune response

$$-y = c \times f\left(\frac{(\text{dose} - b/\text{IPF})}{a}\right) \quad (2)$$

where y is the immune response, $f(x) = e^x/(e^x + 1)$, b is ID_{50} for unprotected groups, IPF equals 1 for unprotected groups, $a < 0$, and c is the maximal immune response. It is assumed that at high doses, there is complete suppression of immune reaction. For this model, the logarithm of dose instead of dose might be used, making the dose curve flatter at high doses and steeper at low doses.

Improved curve modelling (generally because of the non-normality of the distribution of the residuals) was sometimes achieved by transforming the CHS responses before fitting (e.g., square root or log transformation). The relationship between the variance of the CHS measurements and the SSR dose was included in the fitting of the logistic model. Estimates for IPF with their 95% bilateral confidence intervals were calculated directly from the data, and were sometimes improved by bootstrapping.

Suppression of elicitation studies The French group carried out a limited dose–response study (three SSR doses) with the Multitest kit that did not provide sufficient data for an exact IPF determination. It has not been possible to expand the study because the kit is out of production. But, it was possible to determine whether the IPF was equal or not to the SPF by comparing each pre- and post-SSR DTH score with a paired *t*-test. Then, comparisons between sunscreen and non-sunscreen groups were performed on the variation of TSs (pre-SSR−post-SSR) by analysis of variance and Tukey's tests.

The Australian group compared the EI induced by the nickel CHS response (minus the skin background color) at non-SSR exposed sites with the EI at test sites. Statistical significance was assessed by the paired *t*-test to determine the amount of SSR to achieve immunosuppression. The SSR doses that reduced the mean EI of unirradiated skin by 20% were calculated, for both protected and unprotected sites, and were defined as minimal immunosuppressive doses (MISD). The IPF was calculated from the ratio of MISD of protected and unprotected skin using pooled rather than individual volunteer data.

Pros and cons of each method

Suppression of induction studies

Advantages	Local suppression of the induction of CHS occurs at sub-erythemal doses and at doses lower than those needed for suppression of elicitation. It is therefore a very sensitive endpoint, especially for skin types I and II. Its great advantage for sunscreen IPF evaluation is that the duration of SSR exposure is less than those of other methods that require higher or repeated SSR doses.
Disadvantages	This method requires active sensitization of the volunteers. However the allergen (DNCB) is not in any commercial products, so volunteers are unlikely to encounter it again. However if this occurs, the dermatitis is localized and can be easily treated with mild topical steroids.

As it is not possible to test different doses of SSR with and without sunscreen on a single subject, many volunteers are needed so the whole procedure is expensive. This technique takes 3 weeks but in practice the method takes much longer because it is not possible to handle the large number of volunteers needed simultaneously.

Suppression of elicitation responses

Multitest Kit Merieux

Advantages: As with all studies on the elicitation response, this method does not require the sensitization of the volunteers but it is important to verify the initial level of immunity that is very variable. Most people have been immunized in childhood so enrolment of volunteers is easy. The test kit, with a battery of seven antigens, provides comprehensive information on the immune status of the volunteers. Finally, the entire protocol can be completed within 2 weeks.

Disadvantages: The Multitest is about 50 cm^2 so it requires a large SSR exposure area. An acute exposure significantly depress the DTH response but requires doses more than 1 MED. Under these conditions a systemic effect has been observed. This means that SSR dose–response studies with and without sunscreen require different groups of volunteers with each volunteer receiving only one SRR dose with or without the sunscreen. The main disadvantage is that this kit is out of production. However the Mantoux testing with tuberculin purified protein derivative provides a possible alternative model of DTH to a recall antigen.

CHS to nickel

Advantages: This protocol takes advantage of volunteers who have already been sensitized, but a sex bias can be introduced as more women than men are allergic to nickel. The size of the SSR exposed site can be small as the nickel patch diameter is only 9 mm (Finn Chambers). This method enables the determination of SSR-induced immunosuppression dose–responses with and without sunscreen on a single group of volunteers because no systemic effect has been observed with the protocols used. The entire protocol takes 1 week.

Disadvantages: The protocol requires several SSR exposures. Although a recent unpublished study found similar results with a single SSR exposure (T. S. Poon *et al,* personal communication). An acute higher SSR dose may suppress the response but its resulting erythema may mask the nickel-induced erythema CHS reaction. Repeat exposures make it difficult to compare protection afforded by a sunscreen against immunosuppression with SPF that is based on a single exposure. Current protocols have not used individualized MED data but this could be readily done if thought appropriate. At a low concentration of nickel, adapted to the individual sensitivity of the volunteer, the reactions are mild and can be difficult to measure. The pooling of data is required to overcome inter-individual variation.

Discussion Questions and Answers

Which model is more relevant for the prediction of efficacy of sunscreen protection against the development of skin cancer? Animal and human studies that link photocarcinogenesis to UVR-induced immunosuppression are based on the suppression of the induction arm of cell-mediated immunity. The role of suppression of the induction arm is not known. But, as carcinogenesis is a long process with new tumor antigens arising during progression, and tumor antigen concentration increases as tumors develop, it is likely that neither CHS nor DTH accurately mimics antigen exposure during development of anti-tumor immunity.

Which is the best immunological endpoint for IPF assessment: Suppression of induction or elicitation arms of the responses? A definitive answer is not possible at present. Different mechanisms are probably involved in these immune responses, although this has received insufficient research attention. Both methods are suitable from a technical point of view.

Which method will be the best to evaluate CHS response? Different clinical and quantitative methods, such as diameter, area, color, edema, ultrasound, and SFT, can be used; some of which were found to give similar results by the Austrian and US groups. Routine testing is best conducted with simplified protocols that may include one quantitative method in addition to a clinical score.

Must comparisons of IPF and SPF be made with the same methods, volunteers, SSR sources and body sites? Comparisons of IPF and SPF must be made with similar protocols. When IPF is determined from dose–response analyses on different groups of volunteers, protection against erythema should also be quantified in parallel, based on the entire SSR dose–response. The erythema protection factor obtained cannot be called an "SPF," as this term has been defined by regulatory bodies (FDA and Colipa). The name Ery-PF has been proposed by the UK group (Fourtanier *et al*, 2000; Kelly *et al*, 2003), which found this value was comparable with the SPF.

Should SSR doses, in IPF determination, be fractions or multiples of individual MED or physical doses (J per

cm^2) without taking into account individual SSR sensitivity? The SSR dose range must be defined by taking into account the volunteers' skin type and MED from experimental, published, or historical data. The dose given to sunscreen-treated groups should be that given to the untreated volunteers (MED or J per cm^2) multiplied by the SPF. The SPF may be the mean SPF determined in a different group of comparable volunteers, or the individual SPF measured on each volunteer included in the experimental groups as was carried out by the US group. This approach, however, requires considerably more work.

None of the groups had any experience with a design based on arbitrary SSR doses (e.g., within a tolerable range with arithmetic or geometric progression), making it difficult to evaluate this type of protocol. The statisticians, however, thought that this approach would be more valuable and easier.

The choice of SSR dose in the nickel CHS studies should be based on the considerations given above, bearing in mind that four repeated exposures were used to suppress the response. Thus, the highest daily SSR must not be greater than 1 MED or 1 MED $\times$ SPF on protected sites. Doses greater than 1 MED are required for the recall antigen DHT studies, which limits the dose range because it is unethical to give doses more than 3 MED or 3 MED $\times$ SPF. Furthermore, edema induced by high SSR doses may compromise the DTH reading.

Is it essential to determine an exact IPF because this index will have limited value for the consumer, and will the regulatory authorities recognize it? The current assessment of IPF with a single SSR exposure is based on the suppression of the induction of CHS. This requires SSR dose–response studies, with and without sunscreen, from which IPF is derived from a global ratio (i.e., over the entire dose–response range) that is different from the threshold approach to SPF assessment. The UK group tested different global models and found similar estimations for IPF, resulting in the conclusion that it is best to use the simplest model that defined the general shape of the generally exponential curves.

As untreated and sunscreen-treated responses can be obtained on a single volunteer in the nickel CHS studies, it may be preferable to use a threshold method similar to that for SPF. The MISD, which is the minimum significant effect that is significantly detectable from pooled data, has been proposed, and the IPF is the ratio of MISD protected/MISD unprotected.

A proposed improvement of the IPF calculation would be to use a global two-stage method (Steimer *et al*, 1984), which consists of first estimating each individual IPF from each individual antigen dose–response curve and then estimating an IPF from all individual IPF, taking into account the individual variability's weighted average.

At present, there is no need to label sunscreens with an exact IPF index. In part, this is because consumers still have problems understanding the SPF number, which has been capped at 30 in some countries (Australia, USA) resulting in, possibly confusing, labels such as "SPF 30 plus." Furthermore, there is an increasing trend for indices of UVA protection with which the consumer is still not fully familiar. Labelling the product with an additional protection index, e.g., IPF, may add to consumer confusion, especially when it is not easy to specify what the exact benefits might be. Growing concerns about the possible adverse effects of solar UVR on immunity, however, may prompt health and cosmetic authorities to require industry to include evidence of immunoprotection, which is as least equivalent to SPF, in application files for new sunscreen products. A pass or fail option, as discussed in following section, could satisfy such a requirement, and would also be faster and much less expensive for the sunscreen industry to adopt.

Even if exact IPF determination is not crucial, the panel thought that the level of protection against immunosuppression should be at least equal to that for erythema. What protocol is best for this assessment? A simplified protocol should be validated against existing methods. In this context, it is worth noting that SPF assessment using a single exposure on sun-protected skin has been proposed by Diffey (2003).

Suppression of induction of CHS Volunteers would be selected on the basis of their SSR MED (J per cm^2) within a narrow range (e.g., 4 ± 0.4 J per cm^2). This range encompasses skin type I, II, and III as previous work showed considerable overlap of SSR MED within different skin types (Harrison and Young, 2002), and so it would be better to select volunteers within defined skin types. After selection, volunteers would be randomly assigned to three different groups receiving either (i) no topical treatment and no SSR exposure, (ii) no topical treatment and d J per cm^2 of SSR, where this dose is sub-erythemal and sub-immunosuppressive in the study population, or (iii) sunscreen-treated group with d J per cm^2 multiplied by the mean SPF, measured on a separate group of volunteers or on these volunteers themselves with the same SSR source. The definition of sub-immunosuppressive would have to be clearly defined and could for example be about 50% suppression of control after determination from dose–response studies under the same conditions. An additional control group could be considered, (iv) sunscreen treatment but no SSR, to assess the effect of topical treatment on sensitization. If the IPF $=$ SPF or IPF $>$ SPF, the group receiving d $\times$ SPF J per cm^2 will be protected for both endpoints, showing no erythema or immunosuppression. If IPF $<$ SPF, the d $\times$ SPF J per cm^2 group will show no erythema but may show immunosuppression.

Data analysis should include a non-inferiority test between non-sunscreen treated and SSR and sunscreen and SSR groups, the objective being to prove that the response in the treated group is at least the same as that in the non-treated group exposed. In addition, it would also be desirable to perform a comparison test between both nontreated groups, with and without SSR, to verify whether d per cm^2 induces a significant immunosuppression. Some simulations showed that a fair power is accessible but a specific experiment is certainly needed for a correct assessment. Groups of 15 volunteers may be sufficient.

A statistical analysis of all data has shown similar IPF when calculations were based on one DNCB concentration, e.g., 12.5 μg per 20 μL. The lower concentrations (0, 3.125,

6.25 μg per 20 μL) gave unreliable data with a high inter-individual variation. The high concentration (25 μg per 20 μL) sometimes induces very severe reactions and is unusable for some subjects.

Thus, it would be better to apply four patches using the same DNCB concentration (e.g., 12.5 μg per 20 μL plus one patch soaked in the vehicle), which would reduce the variability of response. Variability can also be reduced by the use of a quantitative method to measure CHS response after taking the pre-challenge value into account.

Suppression of the elicitation of CHS to nickel A desirable improvement of this technique would be to develop an assay for the nickel CHS reaction that is different from skin redness so that higher single SSR doses could be given. Skin edema by ultrasound is a possible option. If the acute SSR dose required to suppress the response is higher than 2 MED, however, it will be difficult to use this protocol to determine whether SPF = IPF as the exposure times with sunscreen will be long and may induce edema in addition to erythema. The daily doses to unprotected sites have to be lower than 1 MED if repeated SSR doses are essential for suppression. Kuchel *et al* (2002) reported that 4 daily 0.5 MED exposures induced suppression without any confounding erythema, when the CHS assessment was made 72 h after the last SSR exposure. A protocol similar to that described for the suppression of induction would be possible.

Conclusion

More research into the relationship between the modulation of the skin's immunity by solar UVR and human skin cancer is required. In the absence of this knowledge, it is prudent to propose that sunscreen use should not substantially alter the relationship between UVR-induced erythema and immune modulation. This can only be achieved if the SPF and IPF of a sunscreen are comparable, and this relationship should be maintained even when the sunscreen is applied such that it will not achieve the labelled SPF, as is often the case in practice. It is recognized that the basis of current endpoints for IPF assessment is pragmatic and within this context there is a need for a standard method of IPF determination that is simple and robust.

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